OBSERVATIONS ON THE METABOLIC ACTIVITY OF THE SOIL MICROFLORA[1]

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Abstract

The metabolic activity of the soil microflora has been studied manometrically with intact soil at optimum moisture and in the presence of various substrates. It was found that casamino acids were oxidized rapidly after a preliminary lag period. Oxygen uptake with this substrate showed no marked variation when the moisture level of the soil was between 60 and 80% of its moisture holding capacity. Mixtures of various non-nitrogenous substrates such as hexoses, pentoses, disaccharides, and organic acids yielded variable results. Uptake of oxygen by the soil in the presence of casamino acids was completely inhibited by dinitrophenol and azide. A marked decrease in respiratory activity in the soil was observed also when the reaction was lowered to pH 3.5 and 4.5. Considerable variation in gas exchange was obtained with soils which varied widely in fertility, organic matter, and nitrogen content.

Introduction

The necessity for studying soil as an ecological unit for a better understanding of the normal activity of the soil population was frequently emphasized by Winogradsky (16). Although some work was carried out along these lines in the intervening years, major attention was focused on the behavior of specific soil isolates. The recent advent of antibiotics and the subsequent study of soil antagonists in their natural habitat has helped to redirect attention to microbial activity in soil. The introduction of the soil percolation technique by Lees and Quastel (6) with its application to various soil microbiological problems (1, 7, 14) exemplifies this trend in soil microbiology.

More recently the study of soil as a whole has involved the use of a variety of respirometers to measure CO_2 and O_2 exchange. In 1932 Smith and Brown (12) described a modified Barcroft differential manometer for the determination of CO_2 evolution in soil. These authors stated that the rates of O_2 uptake and CO_2 evolution of soils were in the ratio of 1 : 1 and suggested that the two might be used interchangeably, thus confirming Russell's (11) belief that the amount of O_2 absorbed is a reliable criterion of the total activity of soil microorganisms. In 1947 Webley (15) reported on the use of the Warburg manometric technique, and later, Lees (5) described a modified Haldane respirometer for determining O_2 uptake in soil. Various modifications of these manometric techniques have proved extremely useful in the study of the endogenous activity of the soil flora (2, 10), soil aeration (9), nitrification (8), decomposition of herbicides (3), and the effects of air-drying on soil (13).

The desirability of developing a reliable and practical method for the study of metabolic activity in soil has prompted a reinvestigation of the manometric technique. The methods adopted and the results obtained are presented in this paper.

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Preliminary Experiments

Initial attempts to concentrate large numbers of soil organisms into a small volume and thus increase total gas exchange above that which might be expected from a few grams of soil were made in the following manner. Field soil was suspended in *M*/50 phosphate buffer at pH 6.0 (1 part soil : 1.6 parts buffer), and placed in a Waring blendor for one minute. The mixture was allowed to settle for four hours after which the supernatant fluid was siphoned off and centrifuged at 6000 r.p.m. for five minutes. The resulting sediment was resuspended in buffer so that 2 ml. was equivalent to 400 ml. supernatant. Two milliliters of this suspension was used per Warburg vessel and mixtures of various substrates placed in the side arms. Results of these experiments are given in Fig. 1, where the ready availability of casamino acids to the soil flora is evident. Oxygen uptake with this material was much greater and proceeded at a much faster rate than with the other substrate mixtures tested. Although the bacterial count had been increased from 68 million (initial soil count/gm.) to about 5 billion in the final 2 ml. of suspension, a corresponding increase in respiratory activity did not occur. Inasmuch as the final suspension used was extremely viscous and not readily aerated on shaking, it was felt that the lack of oxygen may have been a limiting factor in this system. Because of the time and effort involved in the preparation of these suspensions and the obvious lack of aeration, subsequent experiments were carried out with the soil itself.

Influence of Moisture

In the following studies, soils previously held in a moist condition were rapidly air-dried (30–60 min.) at room temperature with the aid of a fan, sieved, and the 0.5–1.0 mm. fraction collected. It has previously been shown with soils dried for a longer period (12–24 hr.) that anomalous respiratory patterns result during the first few hours following remoistening (13). Four grams of soil was added to each Warburg vessel. Water or a solution of substrate was then added directly to and distributed as uniformly as possible over the soil in the flask. Sufficient solution was used to bring the moisture content of the soil up to 20, 40, 60 and 80% of the moisture holding capacity (m.h.c.). Alkali was then added to the center wells, and the flasks attached to the manometers and placed in the water bath at 30° C. After a 30 min. equilibration period the manometers were closed and readings started. All tests were carried out in duplicate with casamino acids as the substrate.

The results illustrated in Fig. 2 indicate that oxygen uptake of the soil showed no marked variation when the moisture level was between 60 and 80% of its m.h.c. Below 60%, gas exchange decreased with decrease in moisture. A similar experiment carried out with another soil indicated comparatively little change in oxygen consumption between the 40 and 70% moisture levels. Although slight variation in the moisture levels necessary for optimum metabolic activity was noted for different soils, it would appear practical to standardize at the 60–70% level.

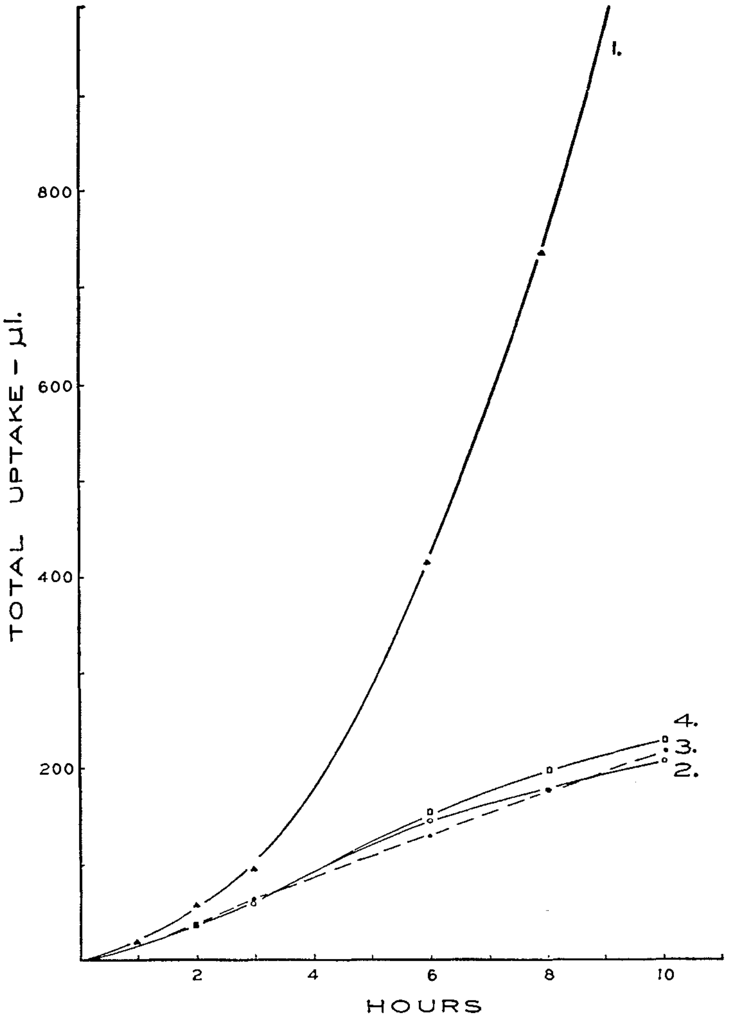

FIG. 1. Oxygen uptake by a concentrated soil suspension. Each Warburg vessel contained: 2.0 ml. soil concentrate, 0.5 ml. substrate, and 0.2 ml. 20% KOH. 1. Casamino acids, 10 mgm. 2. 0.1 ml. each of *M*/20 glucose, fructose, mannose, arabinose, xylose. 3. 0.1 ml. each of *M*/20 lactose, sucrose, maltose, glycerol, mannitol. 4. 0.1 ml. each of *M*/20 acetate, lactate, succinate, fumarate, pyruvate. Values for control (unamended) soil subtracted.

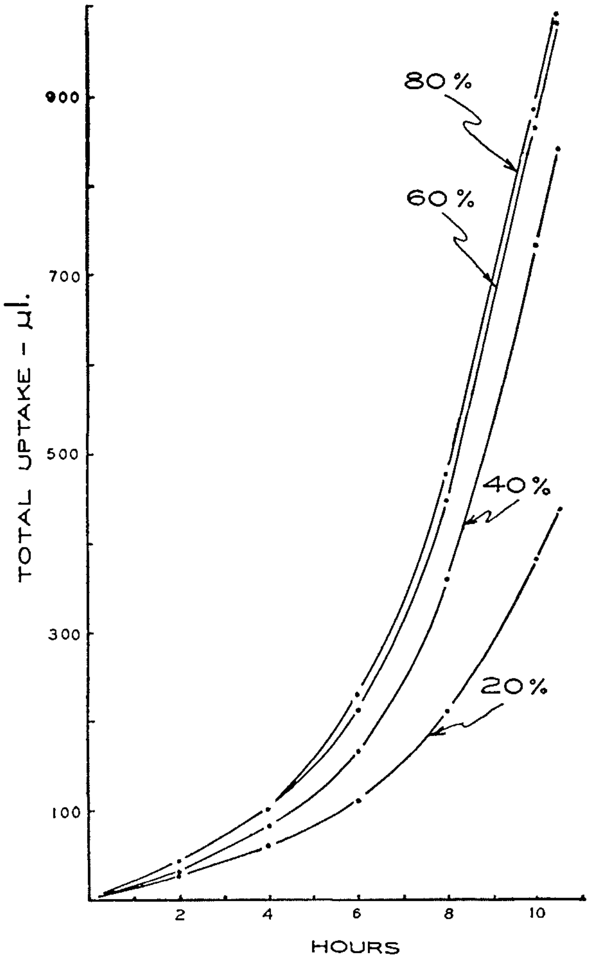

FIG. 2. Oxygen uptake by soil as affected by moisture. Each vessel contained 4 gm. soil, 0.5 ml. casamino acids (10 mgm.), water as indicated in percentage of moisture holding capacity of the soil, 0.2 ml. 20% KOH. Values for control (unamended) soils subtracted.

Effect of Substrate

In addition to casamino acids a number of other substrates were tested to determine the metabolic potential of the soil. Groups of hexoses, pentoses, disaccharides, and organic acids were used in the combinations indicated in Fig. 3. The mixture of all the carbon compounds gave an immediate and rapid response. Of the individual groups, that containing "acids" appeared to be superior to the others. Following a preliminary lag period casamino acids were again oxidized most rapidly. Glutamate oxidation followed a similar pattern except that a longer lag period was noted. When all the groups indicated were combined and one group at a time omitted, oxygen uptake was

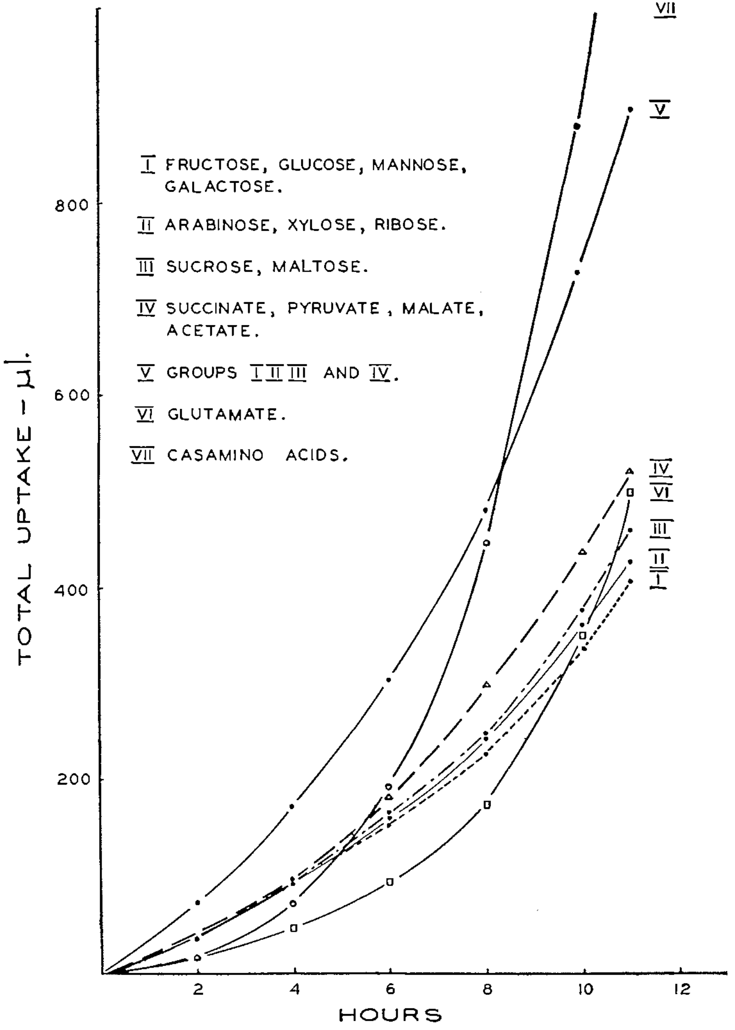

FIG. 3. Oxygen uptake with hexoses, pentoses, disaccharides, organic acids, glutamic acid, and casamino acids. Each vessel contained 4 gm. soil, 0.1 ml. *M*/10 non-nitrogenous substrates as indicated or 0.5 ml. *M*/20 glutamic or 0.5 ml. casamino acids, water to 1.5 ml., 0.2 ml. 20% KOH. Values for control (unamended) soil subtracted.

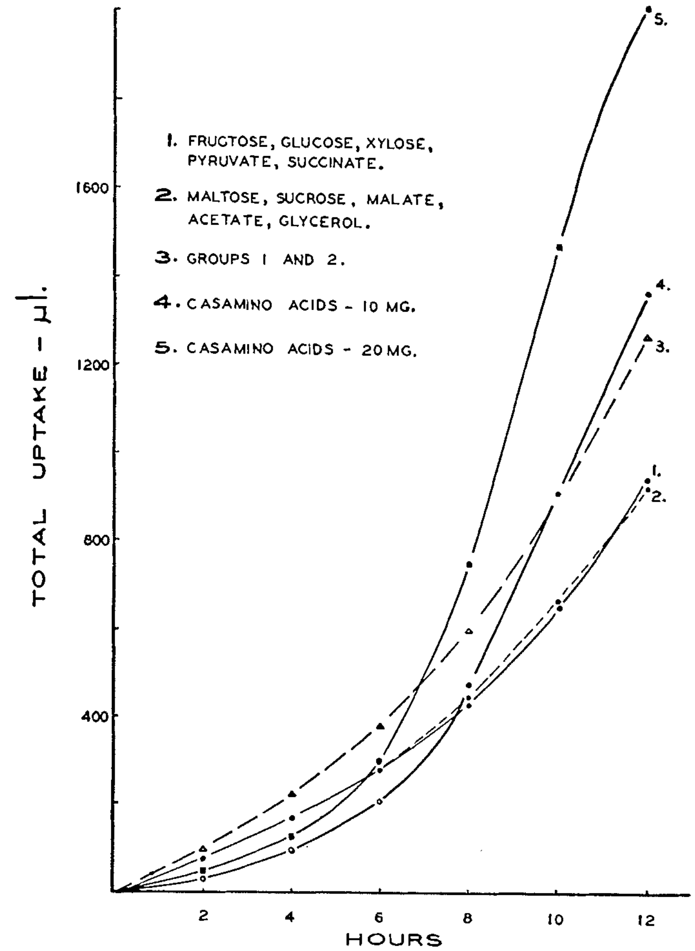

FIG. 4. Oxygen uptake with other mixtures of substrates and with two concentrations of casamino acids. Vessels contained 4 gm. soil, 0.1 ml. $M/10$ substrate as indicated or 0.5 ml. or 1.0 ml. casamino acids (10 or 20 mgm.), water to 1.5 ml., 0.2 ml. 20% KOH. Values for control (unamended) soil subtracted.

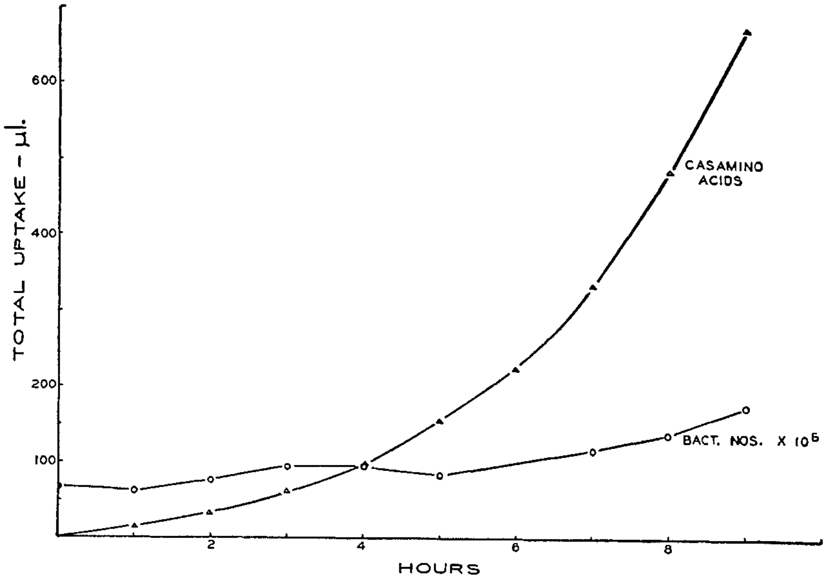

FIG. 5. Oxidation of casamino acids as related to bacterial count. Vessels contained 4 gm. soil, 0.5 ml. casamino acids (10 mgm.), water to 1.5 ml., 0.2 ml. 20% KOH.

lowered appreciably, the most pronounced effect occurring when the organic acids were left out. Combinations of substrates other than the above were tested and essentially similar results obtained as shown in Fig. 4. Doubling of the concentration of amino acids caused a distinct increase in oxygen uptake. A similar effect was observed when the concentration of the other carbon compounds was increased.

In an attempt to relate microbial numbers with oxygen uptake in the presence of casamino acids, soil was incubated with this substrate and samples were plated at hourly intervals in soil-extract agar. In the nine-hour period of the experiment the plate count increased from 60 × 10^6 to 172 × 10^6 whereas the hourly rate of oxygen consumption increased from 0 to 186. Direct counts of these samples showed a doubling of the total number of cells after nine hours. Oxygen uptake and plate counts are illustrated in Fig. 5. The results suggest that the rapid increase in oxygen utilization after a lag period of a number of hours may be due to an adaptive mechanism rather than to multiplication of soil bacteria. The introduction of inhibitors such as 2,4-dinitrophenol (DNP) and sodium azide in a final concentration of $M/2000$ completely inhibited oxygen uptake with casamino acids without appreciably affecting the activity of the unamended soil (Fig. 6). However, oxygen uptake was not inhibited when DNP was introduced four hours after the addition of casamino acids, further supporting the possibility that an adaptive mechanism was operative.

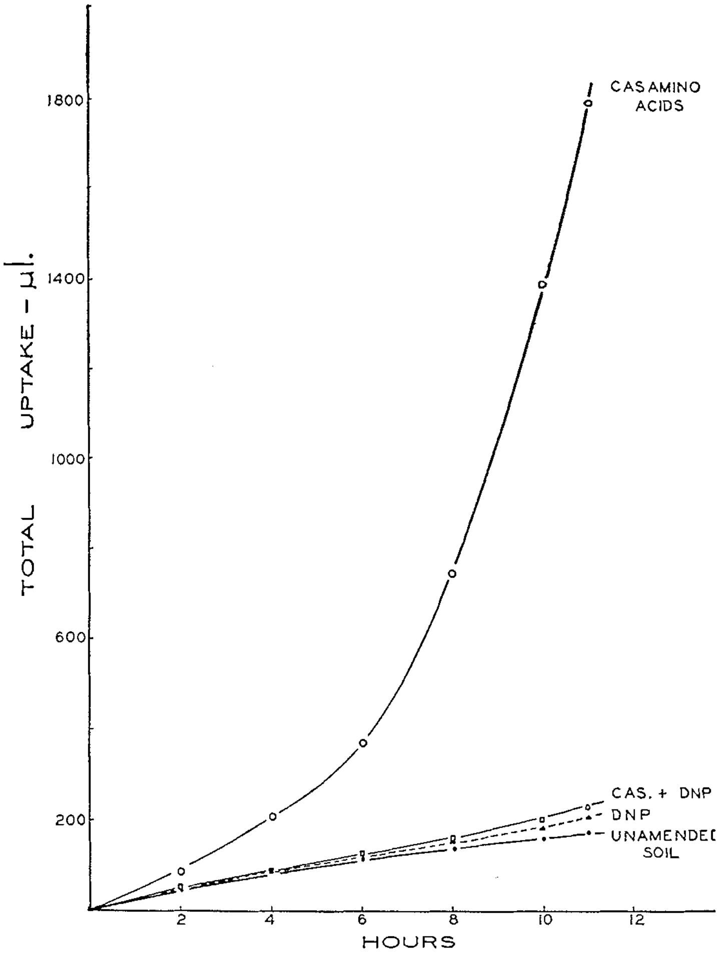

FIG. 6. Inhibition of casamino acid oxidation by 2,4-dinitrophenol. Vessels contained 4 gm. soil, 0.5 ml. casamino acids (20 mgm.), 0.3 ml. *M*/200 2,4-dinitrophenol, water to 1.5 ml., 0.2 ml. 20% KOH.

Influence of Reaction (pH)

In view of the heterogeneity of the soil micropopulation an effort was made to establish that proportion of the respiratory activity attributable to bacteria. Soil reaction was adjusted with HCl to bring the final pH of the moistened soil to 3.5, 4.5, and 5.5; the pH of the unadjusted soil was 6.8. The data presented graphically in Fig. 7 indicate a marked decrease in respiratory activity at pH 3.5 and 4.5, reactions at which bacterial activity is low. At pH 5.5, which is somewhat more favorable for bacteria, the metabolic activity is higher, though still lower than in the unacidified soil.

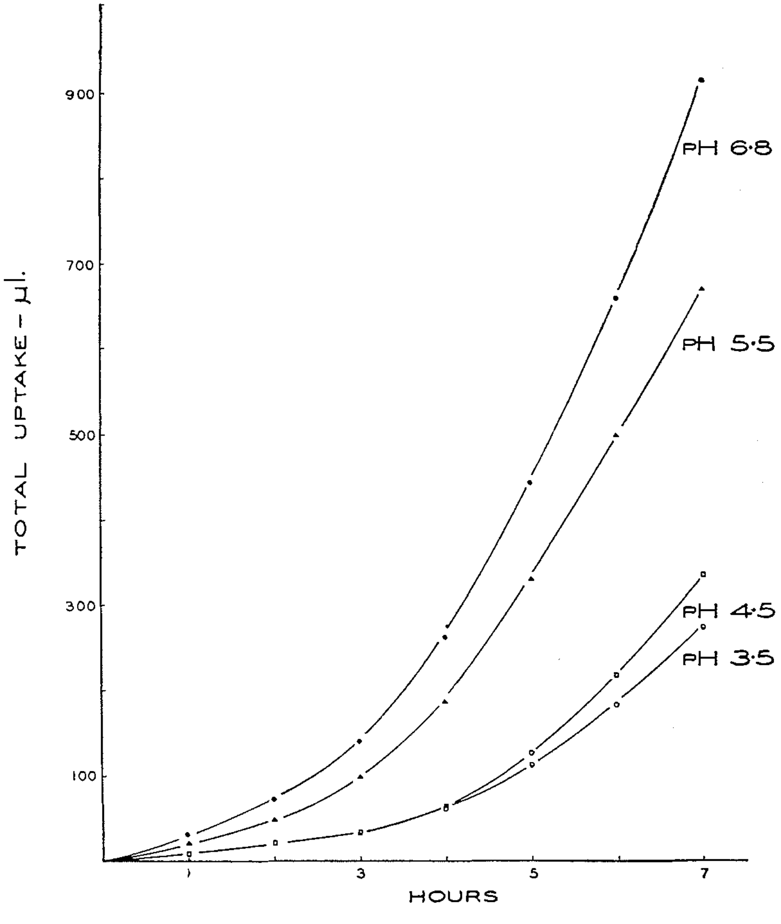

FIG. 7. Influence of soil reaction on oxidation of casamino acids. Vessels contained 4 gm. soil adjusted to pH indicated, 0.5 ml. casamino acids (10 mgm.), water to 1.5 ml., 0.2 ml. 20% KOH. Values for control (unamended) soils subtracted.

Oxidations with Different Soils

The soil used in the above experiments was a comparatively infertile greenhouse soil. Tests were conducted also with a variety of other soils with casamino acids as substrate. As may be seen in Fig. 8, the activity of the unamended soils varied markedly. Soil G, the poorest (greenhouse) soil, had the lowest respiratory activity. Soil N, an unfertilized field soil, somewhat more fertile than G, showed slightly greater activity but not as great as the same soil (X) which has received annual manurial applications. Soil BC is a rich compost soil and NG a soil with high organic matter content. In the presence of substrate a somewhat different picture appeared, except with soils G and N. Soil NG showed less activity than X and was considerably less active than BC.

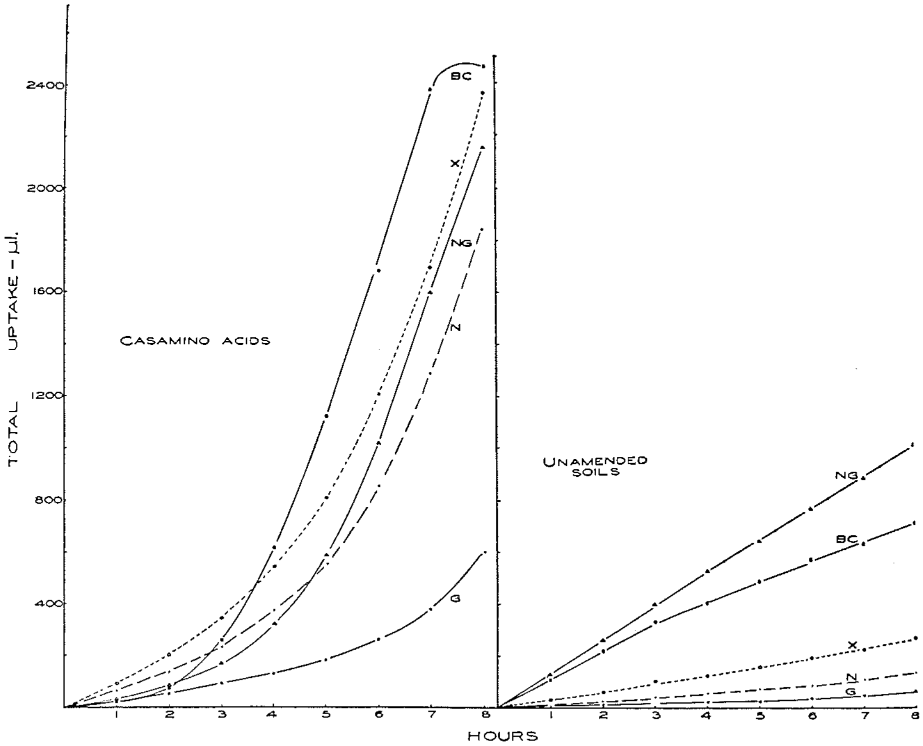

FIG. 8. Metabolic activity of different soils as influenced by casamino acids. Each vessel contained 4 gm. soil and 0.2 ml. 20% KOH. Unamended series: Soils N, X, G received 1.5 ml. water, BC and NG, 2.0 ml. (70% of moisture holding capacity). Casamino acid series: 10 mgm. in same volumes of water as used in unamended series; values for unamended soils subtracted.

Discussion

The foregoing studies indicate the possibility of carrying out manometric studies on soil simply and comparatively rapidly. The use of a suitable substrate may serve to emphasize the activity of the soil population by increasing the reaction rate. However, a fairly reliable indication of the metabolic potential of a soil may also be obtained with the soil alone (Fig. 8). The respiratory activity of such unamended soil will reflect the amount and availability of oxidizable material in the soil, the activity of the population itself and possibly also the treatment it has received. The "autocthonous" flora of a virgin soil may show relatively low metabolic activity whereas a soil which has been cultivated, has received organic matter in various forms, and has a vigorous "zymogenic" population may be very active.

Another point of interest deriving from these experiments is the nature of the response obtained when a substrate such as casamino acids is added to soil. It is suggestive of a typical adaptive reaction, a conclusion which receives some support from the observed inhibition of oxygen uptake by 2,4-dinitrophenol and azide which are known as inhibitors of enzymatic adaptation. That this may represent an adaptive phenomenon is further suggested by the observation that there is no significant change in the number of organisms during the period of oxidation. It is possible, however, that this rapid increase in rate of oxygen uptake is due to the physiological activation of various microbiological elements in the soil (germination of spores of fungi, actinomycetes, and bacteria, etc.) followed by rapid assimilation and oxidation of the substrate. Further studies, including the use of other metabolic inhibitors, may shed light on this phenomenon.

It is clear that the manometric technique for studying respiratory activity in soil has potentialities which warrant further exploration. It holds particular interest as a rapid means of determining the resultant of all the associative and antagonistic forces which are operative in soil, thus permitting the study of soil as an ecological unit. It may facilitate investigations on the activity of various groups of soil organisms (by the use of selective antibiotics) and, when combined with suitable chemical and biochemical methods, may be a useful tool in studies on the nitrogen economy of soil (4), soil fertility, and related problems.

Acknowledgment

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References

1. Audus, L. J. The biological detoxication of hormone herbicides in soil. Plant and Soil, 3 : 170-191. 1951.
2. Chase, F. E. and Gray, P. H. H. Use of the Warburg respirometer to study microbial activity in soils. Nature, 171 : 481. 1953.
3. Gamble, S. J. R., Mayhew, C. J., and Chappell, W. E. Respiration rates and plate counts for determining effect of herbicides on heterotrophic soil microorganisms. Soil Sci. 74 : 347-350. 1952.
4. Greenwood, D. J. and Lees, H. Studies on the decomposition of amino acids in soils. I. A preliminary survey of techniques. Plant and Soil, 7 : 253-268. 1956.
5. Lees, H. A simple apparatus for measuring oxygen uptake of soils. Plant and Soil, 2 : 123-128. 1949.
6. Lees, H. and Quastel, J. H. A new technique for the study of soil sterilization. Chemistry & Industry, 26 : 238-239. 1944.
7. Lees, H. and Quastel, J. H. Biochemistry of nitrification in soil. Biochem. J. 40 : 803-815. 1946.

8. Quastel, J. H. and Scholefield, P. G. Biochemistry of nitrification in soil. Bacteriol. Revs. 15 : 1-53. 1951.
9. Quastel, J. H. and Webley, D. M. The effects of the addition to soil of alginic acid and of other forms of organic matter on soil aeration. J. Agr. Sci. 37 : 257-266. 1947.
10. Rovira, A. D. Use of the Warburg apparatus in soil metabolism studies. Nature, 172 : 29-30. 1953.
11. Russell, E. J. Oxidation in soils and its connection with fertility. J. Agr. Sci. 1 : 261-279. 1905.
12. Smith, F. B. and Brown, P. E. Methods for determination of CO_2 production in soils. Iowa State Coll. Agr. and Mech. Arts, Agr. Expt. Sta. Research Bull. 147 : 27-51. 1932.
13. Stevenson, I. L. Some observations on the microbial activity in remoistened air-dried soils. Plant and Soil. In press. 1956.
14. Stevenson, I. L. and Chase, F. E. Nitrification in an orchard soil under three cultural practices. Soil Sci. 76 : 107-114. 1953.
15. Webley, D. M. A technique for the study of oxygen availability to micro-organisms in soil and its possible use as an index of soil aeration. J. Agr. Sci. 37 : 249-256. 1947.
16. Winogradsky, S. The direct method in soil microbiology and its application to the study of nitrogen fixation. Soil Sci. 25 : 37-43. 1928.